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(54) **Composition and device for in vivo cartilage repair comprising nanocapsules with osteoinductive and/or chondroinductive factors**

Zusammensetzung und Vorrichtung zur Reparatur von Knorpelgewebe in vivo bestehend aus Nanokapseln mit osteoinduktiven und/oder chondroinduktiven Faktoren

Composition et dispositif pour la réparation de cartilage in vivo comprenant des nanocapsules avec des facteurs ostéoinductifs et/ou chondroinductifs

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- **SONG C X ET AL: "FORMULATION AND CHARACTERIZATION OF BIODEGRADABLE NANOPARTICLES FOR INTRAVASCULAR LOCAL DRUG DELIVERY" JOURNAL OF CONTROLLED RELEASE, vol. 43, no. 2/03, 18 January 1997, pages 197-212, XP000632668**

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Description

Background of the Invention:

[0001] Articular cartilage, an avascular tissue found at the ends of articulating bones, has no natural capacity to heal. During normal cartilage ontogeny, mesenchymal stem cells condense to form areas of high density and proceed through a series of developmental stages that ends in the mature chondrocyte. The final hyaline cartilage tissue contains only chondrocytes that are surrounded by a matrix composed of type II collagen, sulfated proteoglycans, and additional proteins. The matrix is heterogenous in structure and consists of three morphologically distinct zones: superficial, intermediate, and deep. Zones differ among collagen and proteoglycan distribution, calcification, orientation of collagen fibrils, and the positioning and alignment of chondrocytes (Archer et al., J. Anat. 189(1): 23-35, 1996; Morrison et al., J. Anat. 189(1): 9-22 1996, Mow et al., Biomaterials 13(2): 67-97, 1992). These properties provide the unique mechanical and physical parameters to hyaline cartilage tissue.

[0002] In 1965, a demineralized extraction from bovine long bones was found to induce endochondral bone formation in the rat subcutaneous assay (Urist Science 150: 893-899, 1965). Seven individual factors, termed Bone Morphogenetic Proteins (BMPs), were isolated to homogeneity and, because of significant sequence homology, classified as members of the TGFβ super-family of proteins (Wozney, et al., Science 242: 1528-34, 1988; Wang et al., Proc. Nat. Acad. Sci. 87: 2220-2224, 1990). These individual, recombinantly-produced factors also induce ectopic bone formation in the rat model (Luyten et al., J. Biol. Chem. 264: 13377-80, 1989; Celeste et al., Proc. Nat. Acad. Sci. 87: 9843-50, 1990). In addition, in vitro tests have demonstrated that both BMP-2 and TGFβ-1 induce mesenchymal stem cells to form cartilage (Denker, et al., Differentiation 59(1): 25-34, 1995; Denker et al., 41st Ann. Orthop. Res. Society 465: 1995). Both BMP-7 and BMP-2 have been shown to enhance matrix production of chondrocytes in vitro (Flechtenmacher J. Arthritis Rheum. 39(11): 1896-904, 1996: Sailor et al., J. Orthop. Res. 14: 937-945, 1996). From these data we can conclude that not only are the BMPs important regulators of osteogenesis, but that they also play crucial roles during chondrogenic development in vitro.

[0003] A partially-purified protein mixture from bovine long bones, termed BP (Bone Protein), also induces cartilage and bone formation in the rat subcutaneous assay (Poser and Benedict, WO95/13767). BP in combination with calcium carbonate promotes bone formation in the body. In vitro, BP induces mesenchymal stem cells to differentiate specifically to the cartilage lineage, in high yields, and to late stages of maturation (Atkinson et al., J. Cellular Biochem. 65: 325-339, 1997).

[0004] The molecular mechanism for cartilage and bone formation has been partially elucidated. Both BMP and TGFβ molecules bind to cell surface receptors (the BMP/TGFβ receptors), which initiates a cascade of signals to the nucleus that promotes proliferation, differentiation to cartilage, and/or differentiation to bone (Massague Cell 85: 947-950, 1996).

[0005] In 1984, Urist described a substantially pure, but not recombinant BMP, combined with a biodegradable polylactic acid polymer delivery system for bone repair (US-4,563,489). This system blends together equal quantities of BMP and polylactic acid (PLA) powder (100 µg of each) and decreases the amount of BMP required to promote bone repair.

[0006] Hunziker (US-5,368,858; US-5,206,023) describes a cartilage repair composition consisting of a biodegradable matrix, a proliferation and/or chemotactic agent, and a transforming factor. A two stage approach is used where each component has a specific function over time. First, a specific concentration of proliferation/chemotactic agent fills the defect with repair cells. Secondly, a larger transforming factor concentration transforms repair cells into chondrocytes. Thereby the proliferation agent and the transforming agent may both be TGFβ differing in concentration only. In addition, the patent discloses a liposome encapsulation method for delivering TFGβ-1 serving as transformation agent.

[0007] Hattersley et al. (WO 96/39170) disclose a two factor composition for inducing cartilaginous tissue formation using a cartilage formation-inducing protein and a cartilage maintenance inducing protein. Specific recombinant cartilage formation inducing protein(s) are specified as BMP-13, MP-52, and BMP-12, and cartilage maintenance-inducing protein(s) are specified as BMP-9. In one embodiment, BMP-9 is encapsulated in a resorbable polymer system and delivered to coincide with the presence of cartilage formation inducing protein(s).

[0008] Laurencin et al., (US-5,629,009) disclose a chondrogenesis-inducing device, consisting of a polyanhydride and polyorthoester, that delivers water soluble proteins derived from demineralized bone matrix, TGFβ, EGF, FGF, or PDGF.

[0009] The results of the approaches to cartilage repair as cited above are encouraging but they are not satisfactory. In particular, the repair tissue arrived at is not fully hyaline in appearance and/or it does not contain the proper chondrocyte organization. Furthermore, previous approaches to cartilage repair have been addressed to very small defects and have not been able to solve problems associated with repair of large, clinically relevant defects.

[0010] One reason that previous approaches failed to adequately repair cartilage may be that they were not able to recapitulate natural cartilage ontogeny faithfully enough, this natural ontogeny being based on a very complicated

system of different factors, factor combinations and factor concentrations with temporal and local gradients. A single recombinant growth factor or two recombinant growth factors may lack the inductive complexity to mimic cartilage development to a sufficient degree and/or the delivery systems used may not have been able to mimic the gradient complexity of the natural system to a satisfactory degree.

[0011] Previous approaches may also have failed because growth factor concentrations were not able to be maintained over a sufficient amount of time , which would prevent a full and permanent differentiation of precursor cells to chondrocytes. The loss of growth factor could be caused by diffusion, degradation, or by cellular internalization that bypasses the BMP/TGFβ receptors. Maintaining a sufficient growth factor concentration becomes particularly important in repair of large sized defects that may take several days or several weeks to fully repopulate with cells.

[0012] The object of this invention is to create a composition for improved cartilage repair in vivo. The inventive composition is to enable in vivo formation of repair cartilage tissue which tissue resembles endogenous cartilage (in the case of articular cartilage with its specific chondrocyte spatial organization and superficial, intermediate, and deep cartilage zones) more closely than repair tissue achieved using known compositions for inducing cartilage repair. A further object of the invention is to create a device for cartilage repair which device contains the inventive composition.

[0013] This object is achieved by the composition and the device as defined by the claims.

Brief description of the invention

[0014] The inventive composition basically consists of a naturally derived osteoinductive and/or chondroinductive mixture of factors (e.g. derived from bone) or of a synthetic mimic of such a mixture combined with a nanosphere delivery system. A preferred mixture of factors is the combination of factors isolated from bone, known as BP and described by Poser and Benedict (WO 95/13767). The nanosphere delivery system consists of nanospheres defined as polymer particles of less than 1000 nm in diameter (whereby the majority of particles preferably ranges between 200-400 nm) in which nanospheres the combination of factors is encapsulated. The nanospheres are loaded with the mixture of factors in a weight ratio of 0.001 to 17% (w/w), preferably of 1 to 4% (w/w) and have an analytically defined release profile (see description regarding Figure 2) showing an initial burst of 10 to 20% of the total load over the first 24 hours and a long time release of at least 0.1 per day during at least seven following days, preferably of 0.1 to 1% over the following 40 to 60 days. The nanospheres are composed of e.g. (lactic acid - glycolic acid)-copolymers (Poly-(D, L)lactic acid-glycolic acid) made of 20 to 80% lactic acid and 80 to 20% of glycolic acid, more preferably of 50% lactic acid and 50% of glycolic acid.

[0015] The loaded nanospheres are e.g. made by phase inversion according to Mathiowitz et al. (Nature, 386: 410-413, 1997) or by other methods known to those skilled in the art (Landry, Ph.D Thesis, Frankfurt, Germany).

[0016] The inventive composition is advantageously utilized as a device comprising any biodegradable matrix including collagen type I and II, and hyaluronic acid in which matrix the nanospheres loaded with the factor combination is contained. The matrix can be in the form of a sponge, membrane, film or gel. The matrix should be easily digestible by migrating cells, should be of a porous nature to enhance cell migration, and/or should be able to completely fill the defect area without any gaps.

[0017] It is surprisingly found that the inventive composition consisting of an osteoinductive and/or chondroinductive combination of factors (e.g. derived from natural tissue) encapsulated in nanospheres as specified above, if applied to a defect area of an articular cartilage, leads to the transformation of virtually all precursor cells recruited to the repair area to chondrocytes, and furthermore, leads to a homogenous chondrocyte population of the repair area and to a chondrocyte order and anisotropic appearance as observed in endogenous hyaline cartilage. These findings encourage the prospect that the inventive composition may lead to significant improvements also regarding repair of large defects.

[0018] As mentioned above, instead of an osteoinductive and/or chondroinductive mixture of factors derived from bone (BP), the inventive composition may comprise natural factor mixtures derived from other tissues (e.g. cartilage, tendon, meniscus or ligament) or may even be a synthetic mimic of such a mixture having an osteoinductive and/or chondroinductive effect. Effective mixtures isolated from natural tissue seem to contain a combination of proliferation, differentiation, and spatial organizing proteins which in combination enhance the tissue rebuilding capacity more effectively than single proteins (e.g. recombinant proteins).

[0019] The specified, analytically defined release profile of such factor mixtures from nanospheres results in the formation of concentration gradients of proliferation and differentiation factors, which obviously mimics the complex gradients of factors observed during natural development very well. The nanosphere extended release profile is sufficient to provide growth factor during the time frame that repair cells arrive into the matrix. The release profile obviously leads to a homogenous population of a matrix with precursor cells, to full differentiation of virtually all of the precursor cells to chondrocytes, and to the formation of an endogenous hyaline cartilage structure.

[0020] Another advantage of the inventive composition is that when the nanospheres are placed in a matrix to form a device for cartilage repair, they are randomly distributed and remain in place when in a joint cartilage defect. During cellular infiltration and differentiation, the nanospheres are in the correct position over the correct time frame.

[0021] Nanospheres have been demonstrated to adhere to the gastrointestinal mucus and cellular linings after oral ingestion (Mathiowitz et al., Nature, 386 410-413 1997). We envisage that nanospheres also adhere to cartilage precursor cells and furthermore, may also adhere to BMP/TGFβ receptors located on the cell membrane. This property allows localized high-efficiency delivery to the target cells and/or receptors. Because of the nanosphere small size and the chemical properties, they are more effective than liposomes or diffusion controlled delivery systems. The efficient delivery to the receptors will facilitate chondrogenesis.

[0022] Derived from the above findings, we envisage the following mechanism for cartilage repair using the effect of the inventive composition. During the first 24 hours (initial burst) 10 to 20% of the total load of the factor mixture is released from the nanospheres into the matrix and diffuses into the synovial environment. Following the initial burst, the nanospheres begin to release factors at a slow rate, which produces gradients of proliferation, differentiation, and spatial organizing proteins. In response to such gradients, precursor cells migrate to the defect site. The loaded nanospheres adhere to cartilage precursor cells and to the BMP and TGFβ receptors to provide localized highly efficient delivery. The precursor cells become differentiated to chondrocytes and secrete type II collagen and cartilage-specific proteoglycans. The composition of the present invention stimulates differentiation of virtually all of these cells to overt chondrocytes and induces an ordered cartilage structure which closely resembles hyaline cartilage. Furthermore, we envisage that this release system will allow homogenous repair of large defect sites and repair of defects from patients with low quantities of precursor cells.

[0023] For in vivo cartilage repair, the inventive device consisting of a matrix and the loaded nanospheres is placed in a chondral lesion that was caused by trauma, arthritis, congenital, or other origin. The damage can result in holes or crevices or can consist of soft, dying, or sick cartilage tissue that is removed surgically prior to implantation of the device. Because of the unique properties of the inventive device precursor cells populate the matrix, differentiate to chondrocytes, and form hyaline cartilage.

[0024] Application of the inventive composition (without matrix) e.g. by injection can be envisaged also, in particular in the case of small defects. Thereby at least 2µg of the composition per ml of defect size is applied or at least 20ng of the osteoinductive and/or chondroinductive mixture encapsulated in the nanospheres is applied per ml defect size.

[0025] The inventive composition and the inventive device are suitable for repair of cartilage tissue in general, in particular for articular cartilage and for meniscus cartilage.

Brief description of the Figures:

[0026] The following figures illustrate the physical and chemical parameters of the inventive composition, the in vitro cartilage inductive activity of BP released from nanospheres and in vivo repair of an articular cartilage defect using the inventive device.

Figure 1 shows a scanning electron micrograph of BP-loaded nanospheres;

Figure 2 shows the release profile (cumulative release vs. time) of the inventive composition;

Figure 3 shows the release profile of the inventive composition compared with release profiles of nanosphere delivery systems loaded with other proteins;

Figure 4 shows the volume of a cartilage defect vs. the days required for populating the defect with repair cells;

Figure 5 shows micromass cultures in the presence or absence of nanospheres loaded with BP;

Figure 6 shows cartilage marker analyses for in vitro cultures containing BP only and for similar cultures containing the inventive composition;

Detailed description of the invention:

[0027] **Figure 1** shows a scanning electron micrograph of BP-loaded nanospheres. The microparticle sizes range from 100-1000 nm with the majority of individual particles ranging between 200-400 nm.

[0028] The release rate profile of the inventive composition was determined by in vitro analysis of BP delivered from nanospheres. These nanospheres were made by phase inversion according to the method as disclosed by Mathiowitz et al. (Nature 386, 410-414, 1997) of ((DL)lactic acid / glycolic acid)-copolymer containing the two acids in a weight ratio of 50:50 and they were loaded with 1% and with 4% of BP.

[0029] For determination of the release rate profile, the nanospheres were placed in a sterile saline solution and incubated at 37°C. BP released into the supernatant was measured using a BCA assay (Pierce). BP released from

the nanospheres as specified shows two successive and distinct profile parts: a fast release (initial burst) of approximately 10 to 20% of the loaded BP over the first 24 hours and a slow release of 0.1 to 1% per day (cumulative 40% to 50%) over 40 to 60 days (**Fig. 2**).

[0030] The release is intermediate between zero-order and first-order kinetics. Both the 1% and 4% encapsulated BP have similar release profiles.

[0031] For attaining release rate profiles as specified above and as necessary for the improved results in cartilage repair the nanospheres are to be adapted accordingly when using factor mixtures other than BP. Thereby, e.g the composition of the nanosphere copolymer, the molecular weight of the polymer molecules and/or the loading percentage of the nanospheres may be changed. The optimum nanosphere character for each specific case has to be found experimentally whereby the release rate profile is analyzed in vitro as described above.

[0032] In the same way, the nanosphere delivery system can be modified regarding the percentage of BP to be released in the first 24 hours, percentage of BP to be released after 24 hours and/or length of time after the first 24 hours during which the remainder of BP is released. In addition, the percentage of BP loaded to the nanospheres is of course variable too, whereby for obtaining the results as described for the specified composition, all the modifications are to be chosen such that the resulting delivery keeps within the range as specified.

[0033] All of the above parameters can be modified to account for the patients age, sex, diet, defect location, amount of blood present in the defect, and other clinical factors to provide optimal cartilage repair. For example, nanospheres with longer release rates are used for treating larger defects and/or for patients with fewer precursor cells (e.g. older patients or patients with degenerative symptoms). In contrast, patients with larger quantities of progenitor cells and/or smaller defects may require a shorter release rate profile.

[0034] **Figure 3** shows the release profile as shown in Fig. 2 for nanospheres as specified above loaded with BP and with other proteins (same loading percentages) such as BSA (bovine serum albumin) or lysozyme. The drastically different release characteristics shows that the profile is dependent on the protein type also. The same is valid for a more hydrophobic mixture of bovine bone derived proteins (PIBP).

[0035] Figure 3 illustrates the singularity of the inventive combination consisting of the specific delivery system (nanospheres as specified above encapsulating the factors) and the specific protein mixture (BP) which is obviously the key to the improved results in cartilage repair as observed when using the inventive composition or device.

[0036] To determine the length of time required for precursor cell repopulation of different sized defects, the following calculation was performed. We estimate that approximately 50,000 cells are recruited to the defect/day. Since the cellular density of cartilage is about 4×10^7 cells/ml, a 10 µl volume defect will take approximately 8 days to fill with cells. **Figure 4** plots the number of days required to fill different volume defects with cells. The Figure assumes an infinite supply of cells and a constant rate of cell attraction to the defect site. The graph demonstrates that the larger a defect size is, the more time is required to completely fill it with cells. Since a 60 µl volume defect will take over 45 days to fill, this Figure demonstrates the necessity for a long term release of factors to induce differentiation of the precursor cells over up to a two month period.

[0037] To determine whether BP bioactivity is harmed by the encapsulation process and to determine whether the released BP was fully bioactive, the following assay was performed. Previously, it was demonstrated that 10T1/2 micromass cultures exposed to BP induce formation of a three dimensional spheroid structure that can be observed macroscopically in tissue culture wells (Atkinson et al., J. Cellular Biochem. 65: 325-339, 1997). BP concentrations equal or greater than 20 ng/ml were required for spheroid formation. No spheroid forms in the absence of BP or at concentrations less than 10 ng/ml (see following table). In this assay, 10T1/2 mesenchymal stem cells act as in vitro models for the precursor cells recruited to a natural defect

[0038] We employed the same assay to test the bioactivity of BP released from 1% loaded nanospheres. BP was eluted from nanospheres at 37°C in a 5% CO_2 humidified incubator. After 24 hours 16% BP is released; and between 24 hours and 7 days, 7% BP was released (Fig. 2). The supernatant was collected, serial dilutions were made, and the supernatant was added to 10T1/2 micromass cultures. BP released from nanospheres at both time points formed spheroids at concentrations greater than 20 ng/ml, but not at concentrations between 0 and 10 ng/ml (see following table). Non-encapsulated BP also formed spheroids at concentrations greater than 20 ng/ml, but not at concentrations between 0 and 10 ng/ml. We conclude that both nanosphere encapsulation and release of BP does not inhibit BP bioactivity.

Spheroid formation (- = no spheroid formation; + = spheroid formation):		
state of used BP	BP concentration (ng/ml)	
	0 - 10	20 - 1000
non-encapsulated BP	-	+
released from nanospheres (24 h)	-	+

(continued)

Spheroid formation (- = no spheroid formation; + = spheroid formation):		
state of used BP	BP concentration (ng/ml)	
released from nanospheres (168 h)	-	+

[0039] To determine the effect of BP slow release in the direct presence of micromass cultures, the following assay was performed. Nanospheres were washed for 24 hours and the supernatant was discarded. The nanospheres were then added to micromass cultures at a quantity such that 10 or 25 ng/ml of BP would be released over 24 hours. Release of 25 ng/ml resulted in spheroid formation whereas release of 10 ng/ml did not form spheroids (**Fig. 5**). Similarly, the addition of 10 ng of non-encapsulated BP per ml did not form a spheroid whereas the addition of 25 ng of non-encapsulated BP per ml did form a spheroid. Regarding the specific in vitro set -up, we conclude that slow release of BP over 24 hours is as effective as a single dose of BP.

[0040] To determine whether the BP released from nanospheres was as chondrogenic as non-encapsulated BP, spheroids were analyzed for type II collagen and proteoglycan content. 10T1/2 spheroids from the above assay that had formed with 1 µg of released BP per ml or 1 µg of non-encapsulated BP per ml were tested histologically with Azure and H+E stains and immunocytochemically with antibodies to type II collagen after 7 days. Both encapsulated and non-encapsulated BP induced cartilage markers such as type II collagen, proteoglycan, and round cell shape (**Fig. 6**). In addition, no qualitative differences were observed between encapsulated and non-encapsulated BP with respect to cell quantity, viability, morphology, or organization (Fig. 6). We conclude that BP retains full chondrogenic capacity after release from nanospheres.

[0041] The in vitro models used for determining the chondroinductive effect of BP differ from the in vivo case by the fact that in the in vitro case the precursor cells are present in an appropriate number and in an appropriate distribution whereas in the in vivo case the precursor cells first have to populate the defect and for this reason have to migrate into the defect. Only in the latter case and for achieving repair cartilage which resembles natural cartilage to a high degree, it is essential for the BP to be released over a prolonged time period according to a specific release profile.

EXAMPLE

[0042] The following example shows that BP released from nanospheres induces cartilage repair in chondral defects in vivo whereby virtually all cells recruited to the defect become chondrocytes, whereby the cell structure obtained is ordered, and whereby a hyaline matrix is built up.

[0043] Using a sheep model, unilateral defects of 0.5mm width, 0.5mm depth and 8 to 10mm length were created in the trochlear groove of the patella. The defects did not penetrate the subchondral bone. The sheep employed in this study were seven years old and displayed degenerative symptoms, including brittle bones, chondromalacia, and subchondral cysts. Because of their advanced age and degenerative symptoms, these amimals probably have decreased numbers of precursor cells. The defects were then dressed according to Hunziker and Rosenberg (J. Bone Joint Surg. 78A(5): 721-733, 1996) with minor changes. Briefly, after enzymatic proteoglycan removal with Chondroitinase AC, 2.5µl of a solution containig 200 units Thrombin per ml was placed in the defect. Then, a paste was filled into the defect, the paste containing per ml: 60mg Sheep Fibrinogen (Sigma), 88mg Gelfoam (Upjohn) and either 10µg of BP-nanospheres or 10µg of BP-nanospheres plus 80ng rhIGF-1 (R+D Systems).

[0044] The nanospheres used were the nanospheres as specified in the description regarding Figure 2 and they were loaded with 1% (w/w) of BP.

[0045] Assuming that the in vitro determined release rate is approximately the same as for the in vivo case, 1.0 to 2.0µg BP per ml were released during the first 24 hours and approximately 100ng per day for the following approximately 60 days.

[0046] After eight weeks, necropsies were performed. The repaired cartilage histology showed that virtually all of the precursor cells were differentiated to chondrocytes throughout the defect. In addition, there was an ordered cartilage appearance with cells on the top being more flattened morphologically than cells in the center and with the presence of ordered, stacked chondrocytes in the lowest zone. The repaired cartilage was fully integrated into the endogenous tissue. In addition, the cartilage repaired with only BP-nanospheres was not significantly different from the cartilage repaired using BP-nanospheres plus IGF-1.

[0047] In conclusion, these results demonstrate that BP released from nanospheres is sufficient for cartilage repair and that no addintional factor is required (such as e.g recombinant factor IGF-1). Using the inventive device constitutes a one step method for cartilage repair, whereby the nanosphere release of BP is sufficient for differentiation of virtually all of the precursor cells to chondrocytes and for induction of an ordered cartilage structure.

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[0048]

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Claims

1. Composition for inducing in vivo cartilage repair comprising an osteoinductive and/or chondroinductive protein mixture derived from bone, cartilage, tendon, meniscus or ligament or a sythetic mimic of such a mixture encapsulated in nanospheres, wherein the nanospheres are polymer particles having a size of less than 1000 nm and an in vitro analytically determined profile of release into saline solution with an initial burst of 10 to 20% of the total load over the first 24 hours and a long time release of 0.1 and 1% per day during 40 to 70 days and wherein the nanospheres are loaded with an amount of the mixture constituting between 0.001 and 17% of the total weight of the loaded nanospheres.

2. Composition according to claim 1, **characterized in that** the osteoinductive and/or chondroinductive protein mixture is the mixture known as BP (bone protein) derived from demineralized bovine long bones and partly purified.

3. Composition according to claim 2, **characterized, in that** the nanospheres are loaded with between 1 and 4% weight percent of BP.

4. Composition according to one of claims 1 to 3, **characterized in that** the nanospheres consist of ((D,L)lactic acid / glycolic acid)-copolymer containing 20 to 80% of lactic acid and 80 to 20% of glycolic acid.

5. Composition according to one of claims 1 to 3, **characterized in that** the ((D,L)lactic acid / glycolic acid)-copolymer contains 50% of lactic acid and 50% of glycolic acid.

6. Composition according to one of claims 1 to 5, **characterized in that** the nanospheres are made by phase inversion.

7. Device containing the composition according to one of claims 1 to 6 and further comprising a porous biodegradable matrix suitable to be placed in a cartialge defect..

8. Device according to claim 7, **characterized in that** it contains at least 2µg of loaded nanospheres per ml ofthe porous biodegradable matrix.

9. Device according to claim 7, **characterized in that** it contains at least 20ng of the osteoinductive and/or chondroinductive protein mixture per ml of the porous biodegradable matrix.

10. Device according to one of claims 7 to 9, **characterized in that** the porous biodegradable matrix has the form of a sponge, membrane, film or geL

11. Device according to one of claims 7 to 10, **characterized in that** the porous biodegradable matrix consists of collagen type I, collagen type II or hyaluronic acid.

12. Use of the composition according to one of claims 1 to 6 for preparing a device for in vivo cartilage repair.

13. Use according to claim 12, **characterized in that** the cartilage is articular cartilage or meniscus cartilage.

14. Use of the composition according to one of claims 1 to 6 for the manufacture of a medicament for cartilage repair on an animal with a degenerative disease.

15. Use of the device according to one of claims 7 to 11 for the manufacture of a medicament for cartilage repair on an animal with a degenerative disease.

16. Use according to claim 15 where the device is to be placed into the cartilage defect.

17. Use according to claim 16 where the defect is dressed before placing the device.

18. Use according to claim 12 where the composition is to be administered to the cartilage defect.

19. Use according to claim 18 where the composition is to be injected.

20. Use according to claim 18 or 19, **characterized in that** the composition is to be administered in an amount of at least 2µg per ml defect size.

21. Use according to claim 18 or 19, **characterized in that** the composition is to be administered in an amount such that the osteoinductive and/or chondroinductive protein mixture is present in the defect in an amount of at least 20ng per ml defect size.

Patentansprüche

1. Zusammensetzung zum Veranlassen einer in vivo Knorpelwieder-herstellung, welche eine osteoinduktive und/oder chondroinduktive Proteinmischung aufweist, welche aus Knochen, Knorpelgewebe, Sehnen, Meniskus oder Band oder einer synthetischen Nachahmung einer solchen Mischung abgeleitet wird, welche in Nanosphären eingekapselt ist, wobei die Nanosphären Polymerpartikel sind, welche eine Grösse von weniger als 1000 nm und ein analytisch in vitro bestimmtes Freigabeprofil in Salzlösung aufweisen mit einer anfänglichen schnellen Freigabe von 10 der 20% des Gesamtinhalts über die ersten 24 Stunden und eine Langzeitfreigabe von 0,1 und 1% pro Tag während 40 bis 70 Tagen, und wobei die Nanosphären mit einer Menge der Mischung befüllt sind, welche zwischen 0.001 und 17% des Gesamtgewichtes der gefüllten Nanosphären ausmachen.

2. Zusammensetzung nach Anspruch 1, **dadurch gekennzeichnet, dass** die osteoinduktive und/oder chondroinduktive Proteinmischung eine als BP (Knochenprotein) bekannte Mischung ist, welche aus demineralisierten langen Rinderknochen gewonnen und teilweise gereinigt ist.

3. Zusammensetzung nach Anspruch 2, **dadurch gekennzeichnet, dass** die Nanosphären mit zwischen 1 und 4 Gewichtsprozent des BP gefüllt sind.

4. Zusammensetzung nach einem der Ansprüche 1 bis 3, **dadurch gekennzeichnet, dass** die Nanosphären aus ((D,L) Milchsäure/Glycolsäure) - Copolymer bestehen, welches 20 bis 80% Milchsäure und 80 bis 20% Glycolsäure enthält.

5. Zusammensetzung nach einem der Ansprüche 1 bis 3, **dadurch gekennzeichnet, dass** das ((D,L) Milchsäure/Glycolsäure) - Copolymer 50% Milchsäure und 50% Glycolsäure enthält.

6. Zusammensetzung nach einem der Ansprüche 1 bis 5, **dadurch gekennzeichnet, dass** die Nanosphären durch Phasenumkehr hergestellt sind.

7. Einrichtung, welche die Zusammensetzung gemäss einem der Ansprüche 1 bis 6 enthält und desweiteren eine poröse, biologisch abbaubare Matrix aufweist, welche geeignet ist, um in einem Knorpeldefekt angeordnet zu werden.

8. Einrichtung nach Anspruch 7, **dadurch gekennzeichnet, dass** sie zumindest 2 µg der gefüllten Nanosphären pro ml der porösen, biologisch abbaubaren Matrix enthält.

9. Einrichtung nach Anspruch 7, **dadurch gekennzeichnet, dass** sie zumindest 20 ng der osteoinduktiven und/oder chondroinduktiven Proteinmischung pro ml der porösen, biologisch abbaubaren Matrix enthält.

10. Einrichtung nach einem der Ansprüche 7 bis 9, **dadurch gekennzeichnet, dass** die poröse, biologisch abbaubare Matrix die Form eines Schwammes, einer Membran, eines Filmes oder eines Gels aufweist.

11. Einrichtung nach einem der Ansprüche 7 bis 10, **dadurch gekennzeichnet, dass** die poröse, biologisch abbaubare Matrix aus Collagen Typ I, Collagen Typ II oder Hyaluronsäure besteht.

12. Verwendung der Zusammensetzung nach einem der Ansprüche 1 bis 6 zur Vorbereitung einer Einrichtung für die in vivo Knorpelwiederherstellung.

13. Verwendung gemäss Anspruch 12, **dadurch gekennzeichnet, dass** der Knorpel ein Gelenkknorpel oder Meniskusknorpel ist.

14. Verwendung der Zusammensetzung gemäss einem der Ansprüche 1 bis 6 für die Herstellung eines Medikamentes für die Knorpelwiederherstellung bei einem Lebewesen mit einem degenerativen Leiden.

15. Verwendung der Einrichtung gemäss einem der Ansprüche 7 bis 11 für die Herstellung eines Medikamentes zur Knorpelwiederherstellung bei einem Lebewesen mit einem degenerativen Leiden.

16. Verwendung nach Anspruch 15, wobei die Einrichtung in dem Knorpeldefekt anzuordnen ist.

17. Verwendung nach Anspruch 16, wobei der Defekt vor der Anordnung der Einrichtung zu behandeln ist.

18. Verwendung nach Anspruch 12, wobei der Knorpeldefekt mit der Zusammensetzung zu versehen ist.

19. Verwendung nach Anspruch 18, wobei die Zusammensetzung einzuspritzen ist.

20. Verwendung gemäss Anspruch 18 oder 19, **dadurch gekennzeichnet, dass** die Zusammensetzung in einer Menge von zumindest 2 µg pro ml Defektgrösse zu verabreichen ist.

21. Verwendung gemäss Anspruch 18 oder 19, **dadurch gekennzeichnet, dass** die Zusammensetzung in einer Menge zu verabreichen ist, so dass die osteoinduktive und/oder chondroinduktive Proteinmischung in dem Defekt in einer Menge von zumindest 20 ng pro ml Defektgrösse vorhanden ist.

Revendications

1. Composition pour induire une réparation de cartilage in vivo, comprenant un mélange de protéines ostéo-inductives et/ou chondro-inductives dérivant d'os, de cartilage, de tendon, de ménisque ou de ligament, ou une imitation synthétique d'un tel mélange encapsulé dans des nanosphères, dans laquelle les nanosphères sont des particules de polymère ayant une taille inférieure à 1000 nm et un profil déterminé analytiquement in vitro de libération dans une solution salée avec un jaillissement initial de 10 à 20 % de la charge totale sur les premières 24 heures et une libération à long terme de 0,1 et 1 % par jour durant 40 à 70 jours et dans laquelle les nanosphères sont chargées d'une quantité du mélange constituant entre 0,001 et 17 % du poids total des nanosphères chargées.

2. Composition selon la revendication 1, **caractérisée en ce que** le mélange de protéines ostéo-inductives et/ou chondro-inductives est le mélange connu sous l'appellation BP (protéine d'os) dérivant d'os long de bovin déminéralisés et partiellement purifiés.

3. Composition selon la revendication 2, **caractérisée en ce que** les nanosphères sont chargées avec entre 1 et 4 % en poids de BP.

4. Composition selon l'une des revendications 1 à 3, **caractérisée en ce que** les nanosphères consistent en un copolymère d'acide (D,L)-lactique/acide glycolique contenant de 20 à 80 % d'acide lactique et de 80 à 20 % d'acide glycolique.

5. Composition selon l'une quelconque des revendications 1 à 3, **caractérisée en ce que** le copolymère d'acide (D, L)-lactique/acide glycolique contient 50 % d'acide lactique et 50 % d'acide glycolique.

6. Composition selon l'une quelconque des revendications 1 à 4, **caractérisée en ce que** les nanosphères sont réalisées par inversion de phases.

7. Dispositif contenant la composition selon l'une quelconque des revendications 1 à 6 et comprenant en outre une matrice biodégradable poreuse adaptée pour être placée dans un défaut de cartilage.

8. Dispositif selon la revendication 7, **caractérisé en ce qu'**il contient au moins 2 µg de nanosphères chargées par ml de la matrice biodégradable poreuse.

9. Dispositif selon la revendication 7, **caractérisé en ce qu'**il contient au moins 20 ng du mélange de protéines ostéo-inductives et/ou chondro-inductives par ml de la matrice biodégradable poreuse.

10. Dispositif selon l'une quelconque des revendications 7 à 9, **caractérisé en ce que** la matrice biodégradable poreuse a la forme d'une éponge, d'une membrane, d'un film ou d'un gel.

11. Dispositif selon l'une quelconque des revendications 7 à 10, **caractérisé en ce que** la matrice biodégradable poreuse est constituée de collagène de type I, de collagène de type II ou d'acide hyaluronique.

12. Utilisation de la composition selon l'une quelconque des revendications 1 à 6 pour la préparation d'un dispositif destiné à une réparation de cartilage in vivo.

13. Utilisation selon la revendication 12, **caractérisée en ce que** le cartilage est un cartilage articulaire ou un cartilage de ménisque.

14. Utilisation de la composition selon l'une quelconque des revendications 1 à 6, pour la fabrication d'un médicament destiné à une réparation de cartilage sur un animal atteint d'une maladie dégénérative.

15. Utilisation du dispositif selon l'une quelconque des revendications 7 à 11, pour la fabrication d'un médicament destiné à une réparation de cartilage sur un animal atteint d'une maladie dégénérative.

16. Utilisation selon la revendication 15, où le dispositif est destiné à être placé dans le défaut de cartilage.

17. Utilisation selon la revendication 16, où le défaut est soumis à un curetage avant mise en place du dispositif.

18. Utilisation selon la revendication 12, où la composition est destinée à être administrée dans le défaut de cartilage.

19. Utilisation selon la revendication 18, où la composition est destinée à être injectée.

20. Utilisation selon la revendication 18 ou 19, **caractérisée en ce que** la composition est destinée à être administrée en une quantité d'au moins 2 µg par ml de taille de défaut.

21. Utilisation selon la revendication 18 ou 19, **caractérisée en ce que** la composition est destinée à être administrée en une quantité telle que le mélange de protéines ostéo-inductives et/ou chondro-inductives est présent dans le défaut en une quantité d'au moins 20 ng par ml de taille de défaut.

FIGURE 1

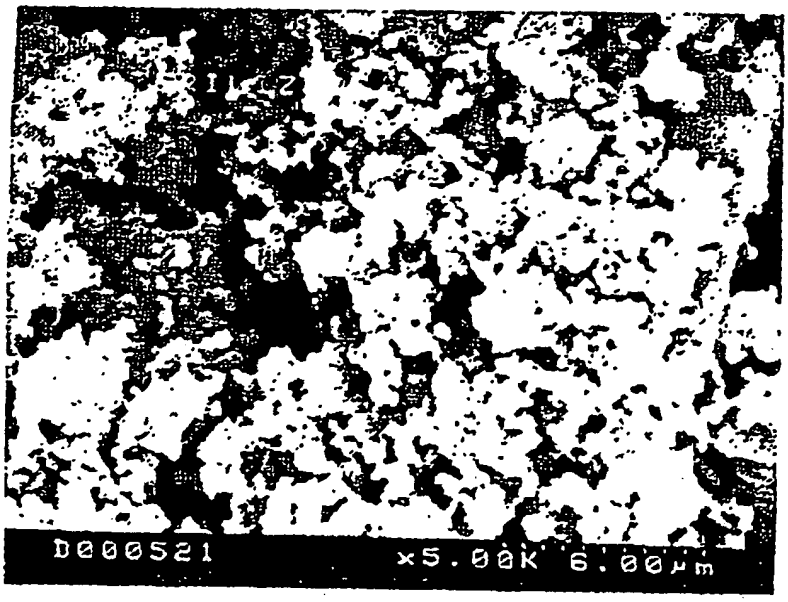

FIGURE 2

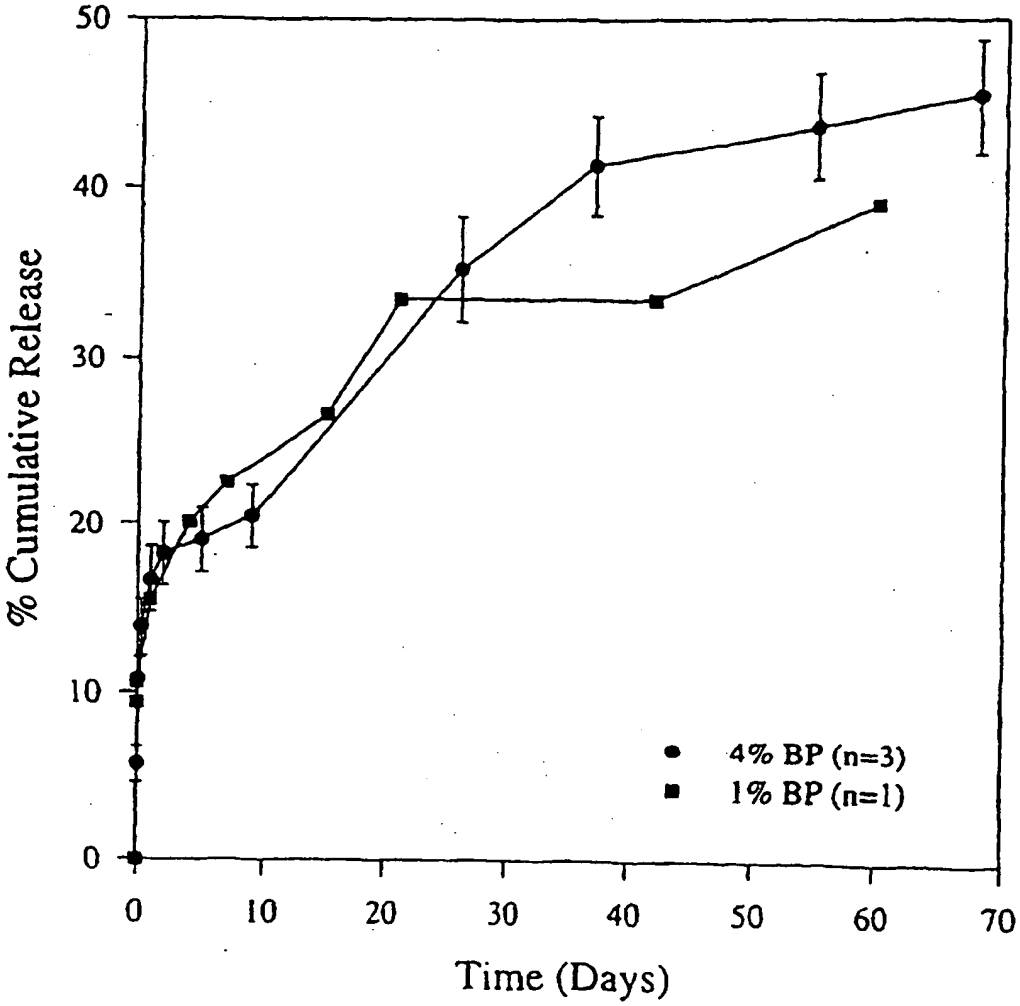
50
40
30
20
10
0
% Cumulative Release
0
10
20
30
40
50
60
70
Time (Days)
4% BP (n=3)
1% BP (n=1)

FIGURE 3

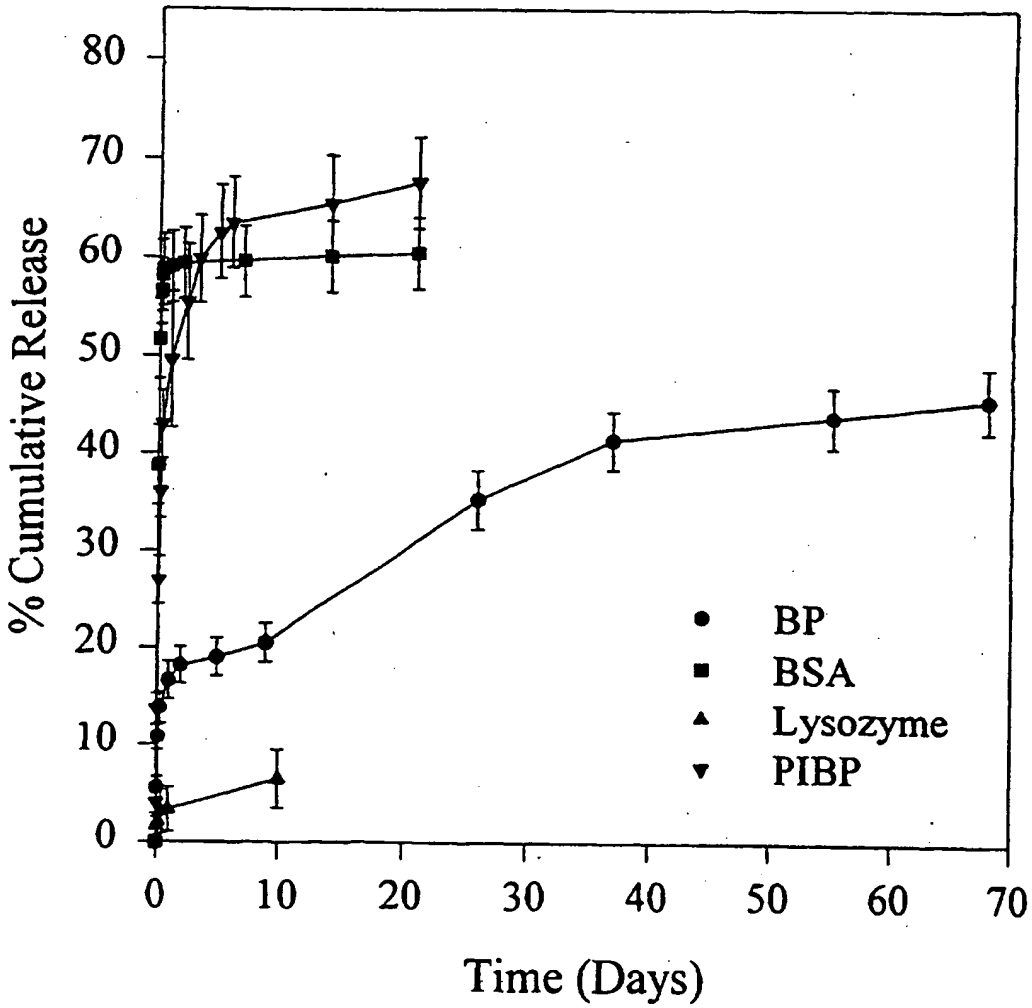
% Cumulative Release
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
Time (Days)
BP
BSA
Lysozyme
PIBP

FIGURE 4

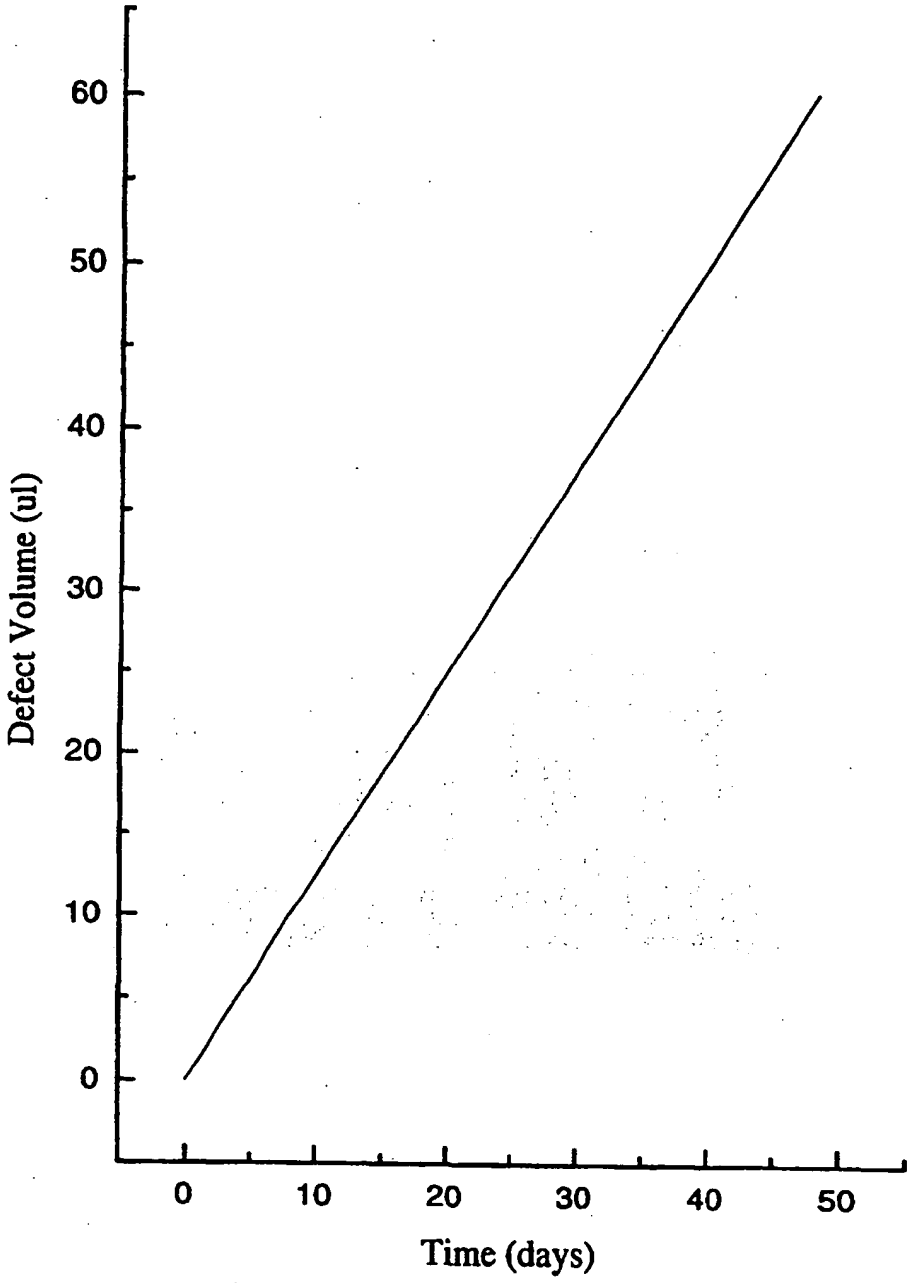

FIGURE 6